Vasodilatory Actions of α-Human Atrial Natriuretic Peptide and High Ca^{2+} Effects in Normal Man

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Abstract

To study vascular actions of synthetic α-human atrial natriuretic polypeptide (αhANP) in man, forearm blood flow (FBF) was measured by strain-gauge plethysmograph during the continuous infusion of 100 ng/min αhANP dissolved in 5% dextrose into the brachial artery in healthy subjects. αhANP increased FBF, with the concomitant increase in ipsilateral limb venous plasma concentrations of αhANP. Overall, there was a significant linear correlation between the decrements of ipsilateral forearm vascular resistance (FVR) during infusions of αhANP and initial FVR levels ($r = -0.883$, $P < 0.01$). Moreover, αhANP, at the stepwise increasing doses of 20, 100, and 500 ng/min, increased FBF in a dose-related fashion: αhANP elicits a concentration-dependent vasodilation of forearm vascular beds. Concomitantly, infusions of αhANP caused a dose-dependent increase in ipsilateral limb venous plasma cyclic guanosine monophosphate (cyclic GMP). Overall, there were direct correlations of FBF either to ipsilateral venous plasma αhANP ($r = 0.724$, $P < 0.01$) or to cyclic GMP concentrations ($r = 0.637$, $P < 0.01$). Subsequently, isoosmolar $CaCl_2$ solution was infused into the same brachial artery at a rate of 0.09 meq/min, and then, with a 2.5±0.2-mg/dl increase in ipsilateral venous serum calcium concentrations the incremental responses of both FBF and plasma cyclic GMP to αhANP were severely blunted. There was also a significant positive linear correlation between FBF and venous plasma cyclic GMP during infusions of αhANP with the simultaneous administration of $CaCl_2$ ($r = 0.807$, $P < 0.01$). Finally, the addition of $CaCl_2$ infusion did not change the slope of the regression line of the FBF-plasma cyclic GMP relationship during infusions of αhANP.

Evidence presented suggests that αhANP acts directly on the forearm vascular beds in man, eliciting its vascular relaxant effect, possibly by increasing cellular levels of cyclic GMP. Moreover, modest elevations of serum calcium inhibit the αhANP-dependent vasodilation, possibly through the suppression of cyclic GMP activation.

Introduction

Mammalian atrial extracts have been demonstrated to contain peptides with potent natriuretic and diuretic properties (1–5). Partial purified atrial extracts were also found to relax isolated vascular smooth muscle (4–6). Several investigators have purified, sequenced, and synthesized atrial peptides (5, 7–10). Recently, the complete sequence of α-human atrial natriuretic polypeptide (αhANP)[1] has been obtained from purified human atrial extracts (11). With the subsequent synthesis of αhANP precise investigation of the renal and vascular actions of this peptide in human beings has been possible. Intravenous administration of αhANP in normal subjects caused marked natriuresis and diuresis (12–20). But, the vasorelaxant action of this peptide has not been defined, despite the occurrence of a mild hypotension (15, 16, 19). In anesthetized rats the infusion of atrial natriuretic peptides (ANP) decreased systemic and regional vascular resistances (21, 22), but in conscious rats and man failed to decrease these vascular resistances (19, 20, 23, 24). Intravenous administration of ANP in conscious animals increased systemic and regional vascular resistances during the fall in blood pressure, possibly through reflex compensatory mechanisms, to maintain blood pressure in the face of the decreased cardiac output (23). In the present study, therefore, we have studied the possible direct effect of αhANP on forearm vascular beds in man, by measurement of forearm blood flow (FBF) followed by the intraarterial administration of synthetic αhANP at such low doses that did not alter heart rate or systemic blood pressure. This method was chosen because it was devoid of circulatory reflexes and so enabled evaluation of direct pharmacological actions on the peripheral vasculature in man (25).

It has been hypothesized that ANP-induced relaxation of vascular smooth muscle may be mediated through the formation of cyclic guanosine monophosphate (cyclic GMP) (26). This hypothesis was based upon the observation that ANP elevated cyclic GMP levels in plasma, vascular smooth muscle, and isolated renal tubules (27–30). Such increases apparently occur through direct activation of particulate guanylate cyclase, rather than inhibition of phosphodiesterase (27, 28). Thus, cyclic GMP may be a second messenger for this hormone (31). It is well established that calcium ion (Ca^{2+}) is an important regulator of hormone-sensitive adenylate cyclase and guanylate cyclase (32). Several investigators have demonstrated that high concentration of extracellular Ca^{2+} blunts the action of antidiuretic hormone, by inhibition of adenylate cyclase activation in the renal tubules (33) and in the toad bladder (34). Moreover, it has been reported that high extracellular Ca^{2+} sharply inhibits particulate guanylate cyclase activity in the heart and lung (35, 36). Therefore, we studied the effect of increasing Ca^{2+} concentration in forearm arterial blood on vasorelaxation and cyclic GMP formation in forearm vascular beds at several concentrations of αhANP.

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1. *Abbreviations used in this paper:* αhANP, α-human atrial natriuretic polypeptide; ANP, atrial natriuretic polypeptide; FBF, forearm blood flow; FVR, forearm vascular resistance.

Methods

All 31 subjects were healthy (5 women, 26 men), young (18–22 yr), Japanese students at University of Tsukuba and were fully informed of the purposes, procedures, and hazards of the experiment; written consent was obtained. These volunteers were studied in the resting, postabsorptive state in an air-conditioned laboratory, with ambient temperature ranging from 24° to 27°C. With the subject comfortable in the supine position and his arms supported at a 45° angle from the long axis of the body, 19-gauge hypodermic needles with attached plastic tubing were inserted in an upstream direction into an antecubital vein of one arm ("ipsilateral" arm) distal to the elbow. Under local xylocaine anesthesia the ipsilateral brachial arteries were also cannulated in an upstream direction with 20-gauge arterial needles. To improve mixing of infusate with brachial arterial blood a jet-injector needle was used for the intraarterial infusions. Infusion rate was 1.0 ml/min (Harvard mini-pump, Harvard Apparatus Co., Natick, MA). This jet-injection system improves mixing of infused substances with brachial arterial blood at infusate kinetic energies too low to cause significant hemolysis. The jet needle was introduced into the brachial artery through the arterial needle, an adaptor on the jet needle hub locking into the hub of the arterial needle. The tip of the jet needle usually protruded up to but not proximal to the intercondylar line at the elbow. To center the jet in the arterial lumen, the position of the jet needle was manipulated to obtain the lowest possible infusion pressure, usually not more than 200 mmHg above pressure during jet infusion into air.

FBF was measured by a plethysmographic technique, as described previously (37, 38). Changes in forearm blood volume were determined by means of a mercury in rubber strain-gauge plethysmograph (39) placed on the midforearm. Strain-gauge was mounted so that its maximal tension was < 10 *g*, to prevent the gauge from obstructing even the superficial veins beneath it. To eliminate the vessels in the hand from these determinations, a sphygmomanometric cuff 7-cm wide was placed around the wrist and inflated to a level exceeding systolic arterial pressure just before each venous occlusion. A sphygmomanometric cuff 13-cm wide was placed around the upper arm, and forearm venous occlusion was produced by suddenly inflating this cuff to a pressure below the diastolic arterial pressure (40 mmHg) (40), utilizing a tank of compressed air to provide a constant pressure source. FBF was taken as the average of four to eight flow measurements made at 15-s intervals. Calculation of FBF was done independently by two of the authors from the copied records, and the average value was used for statistical analysis. The blood pressure was measured in the other arm with a sphygmomanometer. FBF, expressed as milliliters per 100 ml of forearm volume per minute, was calculated from the change in forearm circumference during venous occlusion. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by forearm blood flow (milliliters per 100 ml of forearm volume per minute); these values are expressed as "units" throughout this report. The study consisted of four interrelated protocols (Fig. 1), each of which is described in detail below.

FBF response to αhANP infusion (protocol I; Fig. 1). 16 normal subjects were studied. After placing catheters and a strain-gauge plethysmograph, at least 15 min were allowed for each subject to become accustomed to the study conditions before beginning the protocol. Control solution was composed of 5% dextrose. The experimental solution containing synthetic αhANP was brought to volume with 5% dextrose. With the subject comfortable in the supine position, control solution was infused for 10 min, followed by infusions of solution containing αhANP delivered 100 ng/min into brachial arterial blood for 10 min. During the last 2 min of each infusion, FBF was measured by strain-gauge plethysmograph. Immediately before the αhANP infusion and at the end of the infusion, we sampled the ipsilateral and contralateral venous blood for the measurement of αhANP into the tube containing EDTA · 2Na (1 mg/ml) and Trasylol (500 KIU/ml).

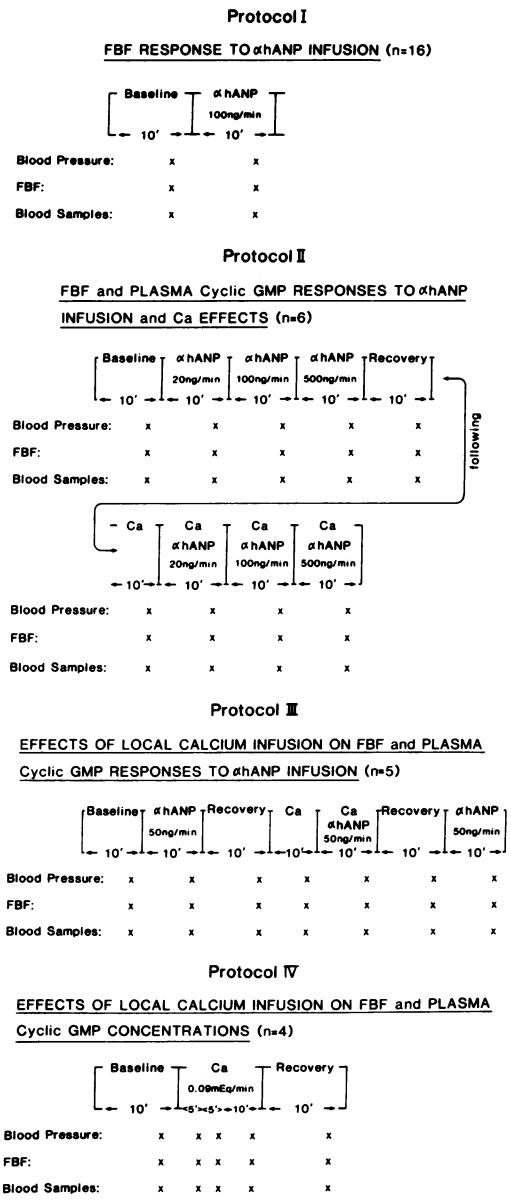

Figure 1. Schematic representation of the protocol design.

FBF and plasma cyclic GMP responses to αhANP infusion and calcium effects (protocol II; Fig. 1). Six normal subjects were admitted to the trial. After the infusion of control solution, αhANP, at stepwise increasing rates of 20, 100, and 500 ng/min, was infused into brachial arterial blood for each 10 min. Subsequently, control solution was again infused for 10 min, and then isoosmolar $CaCl_2$ solution was infused into brachial arterial blood at a rate of 0.09 meq/min for 10 min, followed by infusions of the mixed solutions containing 20 ng/min αhANP and 0.09 meq/min $CaCl_2$ delivered into brachial arterial blood, 100 ng/min αhANP and 0.09 meq/min $CaCl_2$, and 500 ng/min αhANP and 0.09 meq/min $CaCl_2$, respectively. The effect of each infusion of αhANP was compared with forearm hemodynamics measured during a preceding paired infusion of control or $CaCl_2$ solution. As described in protocol I, at each infusion FBF was measured and venous sampling for measurements of calcium, αhANP and cyclic GMP was performed. Plasma samples for the measurement of cyclic GMP were taken with EDTA 2K (1 mg/ml).

Effects of local calcium infusion on FBF and plasma cyclic GMP responses to αhANP (protocol III; Fig. 1). Five normal subjects were

studied. To examine whether the vascular responses to αhANP can be restored to normal after the discontinuation of $CaCl_2$ infusion, the following study was performed. The control solution was infused for 10 min, followed by the infusion of the solution which contains αhANP delivered 50 ng/min into brachial arterial blood. After the infusion of this solution, control solution was infused for 10 min, and then isoosmolar $CaCl_2$ solution was infused into brachial arterial blood at a rate of 0.09 meq/min for 10 min, followed by the infusion of the mixed solution containing $CaCl_2$ and αhANP delivered 0.09 meq/min and 50 ng/min into brachial arterial blood for each 10 min, respectively. Thereafter, control solution was again infused for 10 min, followed by the infusion of αhANP without $CaCl_2$ delivered 50 ng/min into brachial arterial blood. The effect of each αhANP infusion was compared with hemodynamics measured during a preceding paired infusion. As described in protocol I, at each infusion FBF was measured and venous sampling for measurements of calcium and cyclic GMP was performed.

Effects of local calcium infusion on FBF and plasma cyclic GMP concentrations (protocol IV; Fig. 1). Four normal subjects were studied. To examine the effect of high Ca^{2+} on FBF and plasma cyclic GMP, isosmolar $CaCl_2$ was infused into brachial arterial blood at a rate of 0.09 meq/min for 20 min. FBF was measured immediately before the infusion of $CaCl_2$, at 5, 10 and 20 min of $CaCl_2$ infusion, and at 10 min after switching it to the infusion of 5% dextrose. Venous sampling for measurement for cyclic GMP was performed before, at 10 and 20 min during, and at 10 min after the infusion of $CaCl_2$.

Analytical methods. Serum calcium was measured by autoanalyzer. Plasma cyclic GMP was measured by radioimmunoassay (41). Radioimmunoassays of cyclic GMP were done in duplicate after succinylation. Plasma αhANP concentration was determined by radioimmunoassay, as described previously (42). Briefly, radioimmunoassay of αhANP was performed using an antibody generated in NZW rabbits immunized with αhANP conjugated with bovine thyroglobulin. The standard buffer was 0.05 M sodium phosphate buffer, pH 7.4, containing 1% bovine serum albumin (BSA), 0.1% Triton X-100, 0.08 M NaCl, 0.025 M EDTA 2Na, 0.05% NaN_3, and Trasylol 500 KIU/ml. ^{125}I-αhANP was used as a tracer (Amersham Japan, Tokyo, Japan) and synthetic αhANP synthesized in our laboratory (11) was used to construct standard curves. The standard αhANP or the unknown sample (100 μl) was incubated with anti-αhANP antiserum diluent (500 μl) for 12 h at 4°C. Then, the tracer solution (18,000–20,000 cpm in 200 μl) was added. After the incubation for 36 h at 4°C, anti-rabbit IgG goat serum diluent (200 μl) was added. After kept standing for 40 h at 4°C, the tubes were centrifuged at 2,000 × 30 min at 4°C. Using this procedure, the lowest concentration of αhANP yielding a binding significantly different from that in the absence of standard at the 95% confidence interval was 2.5 pg/tube. The 50% intercept was at 59 pg/tube. The interassay variation was 16.8% (n = 6), the intraassay variation 7.4% (n = 10). The antiserum shows 100% crossreactivity with βhANP and γhANP.

Statistical analysis. All data are expressed as the mean±SEM. Analysis variance for repeated measures, with a post-hoc Dunnett's test, was used to compare observed changes versus the baseline (43). One-way analysis variance, with Bonferroni adjustment of the P value, was used when comparing the changes in FBF and plasma cyclic GMP with αhANP before $CaCl_2$ infusion, and after $CaCl_2$ infusion. Paired t test was used to test the significance of single comparisons. Since natural logarithmic transformation rather than absolute values followed a Gaussian distribution, the natural logarithmic transformation of αhANP levels was used for statistical analysis. Regression analysis was performed according to standard procedures. Changes were reported as significant if $P < 0.05$.

Table I. Systemic and Forearm Hemodynamics, and Plasma αhANP Concentrations before and during αhANP Infusion at a Rate of 100 ng/min in 16 Normal Subjects (Mean±SEM)

	Control*	ANP 100
Blood pressure, *mmHg*		
Systolic	114±3	114±4
Diastolic	66±1	65±2
Heart rate, *bpm*‡	61±2	62±3
Forearm blood flow, *ml/100 ml/min*	2.61±0.25	4.82±0.48§
Forearm vascular resistance, *U*	36.8±3.6	18.4±1.6§
Ipsilateral αhANP, *pg/ml*	106±14	649±54§
Contralateral αhANP, *pg/ml*	101±13	107±18

* Control, during the infusion of 5% dextrose; ANP 100, during the infusion of 100 ng/min αhANP.
‡ bpm, beats per min.
§ $P < 0.01$ vs. control.

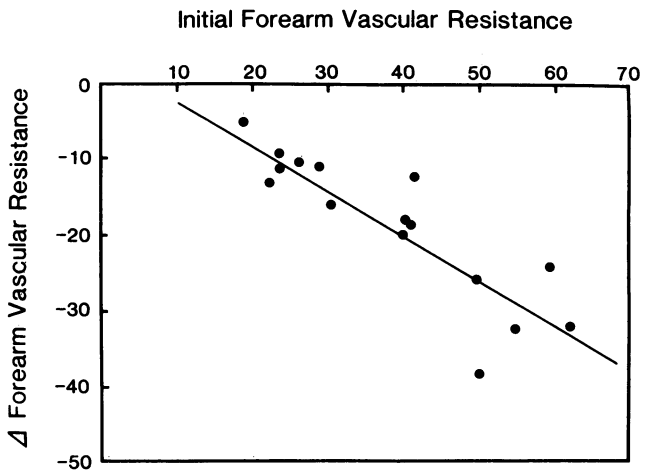

Figure 2. Regression analysis between the decrements of FVR (units) during the infusion of 100 ng/min αhANP and initial levels of FVR (units) in 16 normal volunteers ($r = -0.883$, $P < 0.01$).

Results

FBF response to αhANP infusion. The forearm hemodynamic effects of the intrabrachial arterial infusions of αhANP are presented in Table I. Neither control nor experimental infusions had significant effects on systemic blood pressure or heart rate in normal subjects. Infusion of 100 ng/min αhANP increased FBF from 2.61±0.25 to 4.82±0.48 ml/100 ml per min ($P < 0.01$, by paired t test), and decreased FVR from 36.8±3.6 to 18.4±1.6 U ($P < 0.01$). Concomitantly, ipsilateral venous plasma αhANP concentrations increased from 106±14 to 649±54 pg/ml ($P < 0.01$), without a significant change in contralateral venous plasma αhANP (107±18 vs. 101±13 pg/ml, NS). During infusions of αhANP, systemic blood pressure and heart rate remained unchanged. In all 16 subjects, there was a significant linear correlation between the decrements in FVR during infusions of 100 ng/min αhANP and initial FVR levels ($r = -0.883$, $P < 0.01$) (Fig. 2).

FBF and plasma cyclic GMP responses to αhANP infusion and calcium effects. Infusions of 20, 100, and 500 ng/min αhANP increased FBF from 2.39±0.35 to 3.05±0.33 (NS), 5.23±0.60 ($P < 0.01$) and 6.24±0.64 ($P < 0.01$) ml/100 ml per min, respectively (Table II); FVR decreased from 39.8±6.2 to

Table II. Effects of $CaCl_2$ (0.09 meq/min) on Systemic and Forearm Hemodynamics, and Biochemical Parameters during αhANP Infusions at Rates of 20, 100, and 500 ng/min in Six Normal Subjects (Mean±SEM)

	Control-1*	ANP 20	ANP 100	ANP 500	Control-2	Ca	Ca + ANP 20	Ca + ANP 100	Ca + ANP 500
Blood pressure, *mmHg*	111±3	112±3	110±2	110±2	110±3	110±3	110±2	109±2	109±2
Systolic	66±3	65±3	64±2	64±2	64±2	64±2	64±2	63±3	64±2
Diastolic	59±1	58±1	58±1	58±1	58±1	57±1	57±1	58±1	57±1
Heart rate, *bpm*									
FBF, *ml/100 ml/min*	2.39±0.35	3.05±0.33	5.23±0.60‡	6.24±0.64‡	4.06±0.69	3.06±0.58	2.92±0.56	3.21±0.56	4.15±0.76
FVR, *U*	39.8±6.2	29.8±4.4	17.8±2.9‡	14.7±2.4‡	25.5±5.3	33.9±7.7	40.7±8.8	32.2±6.4	26.4±6.5
Ipsilateral αhANP, *pg/ml*	88±23	134±26	608±48‡	7,160±1,471‡	106±23	96±23	161±30§	743±66§	8,200±1,385§
Contralateral αhANP, *pg/ml*	90±25	92±20	94±22	96±30	100±24	96±24	100±25	101±25	103±32
Ipsilateral cGMP,‖ *pmol/ml*	3.15±1.05	4.45±1.57	5.30±1.51	8.92±3.06§	7.05±1.81	3.57±0.90	3.65±0.77	3.90±1.56	4.41±0.84
Contralateral cGMP, *pmol/ml*	3.20±1.02	3.21±1.12	3.25±1.34	3.35±1.12	3.30±1.32	3.30±0.88	3.32±0.94	3.34±0.86	3.30±1.24
Ipsilateral Ca, *mg/dl*	9.5±0.2	9.4±0.2	9.4±0.2	9.1±0.2	9.2±0.2	11.4±0.3‡	11.9±0.3‡	12.0±0.4‡	12.0±0.3‡
Contralateral Ca, *mg/dl*	9.5±0.2	9.4±0.2	9.4±0.2	9.3±0.2	9.2±0.2	9.4±0.2	9.5±0.2	9.4±0.2	9.5±0.2

* Control-1 and control-2, during the infusions of 5% dextrose; ANP 20, during the infusion of 20 ng/min αhANP; Ca, during the infusion of 0.09 meq/min $CaCl_2$; Ca + ANP 20, during the infusions of 0.09 meq/min $CaCl_2$, and 20 ng/min αhANP. ‡ $P < 0.01$ vs. the value of control-1. § $P < 0.01$ vs. the value of Ca. ‖ Ipsilateral cGMP, ipsilateral venous plasma cyclic GMP concentrations; contralateral cGMP, contralateral venous plasma cyclic GMP concentrations; ipsilateral Ca, ipsilateral serum calcium concentrations; contralateral Ca, contralateral serum calcium concentrations. ¶ $P < 0.05$ vs. the value of control-1.

29.8±4.4 (NS), 17.8±2.9 ($P < 0.01$) and 14.7±2.4 ($P < 0.01$) U, respectively (Table II). Thus, αhANP elicits a concentration-dependent vasodilation of forearm vascular beds. Concomitantly, ipsilateral venous plasma αhANP concentrations increased from 88±23 to 134±26 (NS), 608±48 ($P < 0.01$) and 7,160±1,471 ($P < 0.01$) pg/ml, respectively (Table II). Overall, there was a significant positive correlation between FBF and ipsilateral venous plasma αhANP concentrations ($r = 0.724$, $P < 0.01$) (Fig. 3). Ipsilateral venous plasma cyclic GMP increased from 3.15±1.05 to 4.45±1.57 (NS), 5.30±1.51 ($0.05 < P < 0.1$), and 8.92±3.06 ($P < 0.05$) pmol/ml; thus indicating that αhANP caused a dose-dependent cyclic GMP increase. Moreover, the relaxant effect of αhANP was directly correlated to ipsilateral venous plasma cyclic GMP concentrations ($r = 0.637$, $P < 0.01$) (Fig. 4). During the intrabrachial infusion of $CaCl_2$, FBF was depressed with the accompaning decrease in ipsilateral plasma cyclic GMP (Table II). Ipsilateral venous serum calcium concentrations increased from 9.2±0.2 to 11.4±0.3 mg/dl ($P < 0.01$), but contralateral serum calcium did not change during infusions of $CaCl_2$. Thereafter, infusions of 20, 100, and 500 ng/min αhANP with the simultaneous infusion of $CaCl_2$ did not significantly increase FBF: from a baseline value of 3.06±0.58 to 2.92±0.56 (NS), 3.21±0.56 (NS), and 4.15±0.76 (NS) ml/100 ml per min, respectively (Table II). Thus, the increments of FBF with αhANP infusions were significantly less with the simultaneous infusion of $CaCl_2$ as compared to those without $CaCl_2$: −4.6±3.4% vs. 23.6±10.5% (NS) during the infusion of 20 ng/min αhANP, 8.1±6.7% vs. 133.3±29.0% ($P < 0.05$) during 100 ng/min αhANP, and 36.2±5.7% vs. 181.1±37.1%. ($P < 0.05$) during 500 ng/min αhANP (Fig. 5). Accordingly, with the simultaneous infusion of $CaCl_2$, infusions of 20, 100, and 500 ng/min αhANP did not significantly increase ipsilateral venous plasma cyclic GMP concentrations: from a baseline value of 3.57±0.90 to 3.65±0.77 (NS), 3.90±1.56 (NS), and 4.41±0.84 (NS) pmol/ml, respectively (Table II): the increments of ipsilateral venous plasma cyclic GMP with αhANP were significantly less with the simultaneous infusion of $CaCl_2$ as compared with those without $CaCl_2$: 0.07±0.63 vs. 1.30±0.93 pmol/ml (NS), 0.33±0.62 vs. 2.16±0.64 pmol/ml (NS), and 0.84±0.44 vs. 5.77±1.89 pmol/ml ($P < 0.05$) during infusions of 20, 100, and 500 ng/min αhANP, respectively (Fig. 6). Thus, the simultaneous infusion of $CaCl_2$ blunted the responses of both FBF and plasma cyclic GMP to αhANP. The magnitude of the increases in ipsilateral venous plasma αhANP concentrations during the αhANP infusion did not differ between with and without $CaCl_2$ (Table II). Contralateral venous serum calcium concentrations did not change. Neither did contralateral venous plasma cyclic GMP change. Throughout the study, systemic blood pressure or heart rate did not change (Table II).

Finally, there were significant correlations of FBF either to ipsilateral venous plasma cyclic GMP concentrations during infusions of αhANP without $CaCl_2$ or to cyclic GMP during infusions of αhANP with $CaCl_2$ (Fig. 4). Fig. 4 shows no significant difference in the slope between the two regression lines of the FBF-cyclic GMP relationship during infusion of αhANP. Thus, it is suggested that the presence of modest increases in serum calcium results in a blunting of the vasodilator effects of αhANP as well as a blunting of the secondary increase of cyclic GMP, but does not affect the vasodilator responses to the cyclic GMP increase.

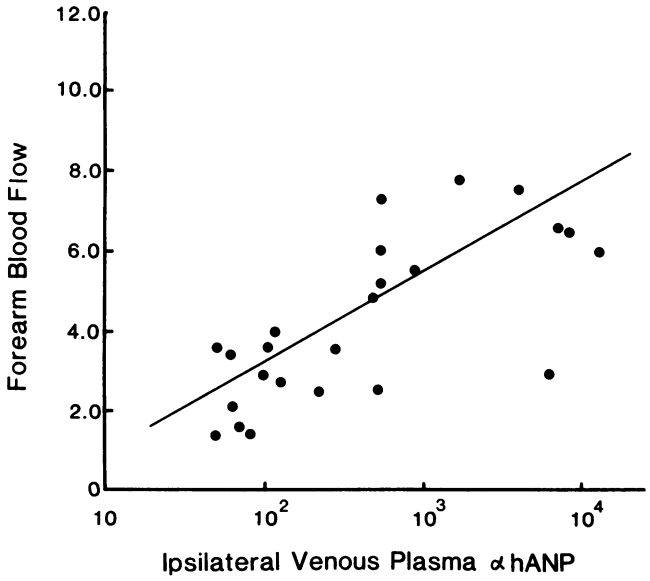

Figure 3. Regression analysis between forearm blood flow (ml/100 ml per min) and ipsilateral venous plasma αhANP concentrations (pg/ml) during the infusions of αhANP at stepwise increasing doses of 20, 100, and 500 ng/min for each 10 min ($r = 0.724$, $P < 0.01$).

Effects of local calcium infusion on FBF and plasma cyclic GMP responses to αhANP. Infusion of 50 ng/min αhANP increased FBF from 2.89±0.75 to 4.21±0.80 ml/100 ml per min ($P < 0.05$) (Table III). The simultaneous infusion of $CaCl_2$ and αhANP caused an insignificant increase in FBF with αhANP: from 1.62±0.39 to 1.78±0.28 ml/100 ml per min (NS). After the discontinuation of $CaCl_2$ infusion, however, αhANP produced the significant increase in FBF again: from 2.33±0.50 to 3.53±0.71 ml/100 ml per min ($P < 0.05$). With the simultaneous infusion of $CaCl_2$, percent increments of FBF during infusions of αhANP were significantly smaller (20.9±15.9%) as compared with those before (58.1±13.5%, $P < 0.05$) and after $CaCl_2$ infusion (55.5±17.4%, $P < 0.05$) (Fig. 7). Infusion of 50 ng/min αhANP increased ipsilateral venous plasma cyclic GMP from 3.20±0.44 to 5.10±0.82 pmol/ml ($P < 0.05$), from 3.90±0.94 to 4.70±0.85 pmol/ml (NS), and from 4.20±0.78 to 5.84±0.67 pmol/ml ($P < 0.05$), before, during and after the simultaneous infusion of $CaCl_2$, respectively (Table III): the increments of cyclic GMP with αhANP were significantly less during the simultaneous infusion of $CaCl_2$ (0.80±0.52 pmol/ml) as compared to both those before $CaCl_2$ infusion (1.90±0.92 pmol/ml, $P < 0.05$, by paired t test) and those after $CaCl_2$ infusion (1.64±0.64 pmol/ml, $P < 0.05$). Thus, the administration of $CaCl_2$ inhibited the vascular actions of αhANP, and after the discontinuation of $CaCl_2$ infusion the blunted responses of FBF and cyclic GMP to αhANP were restored to normal.

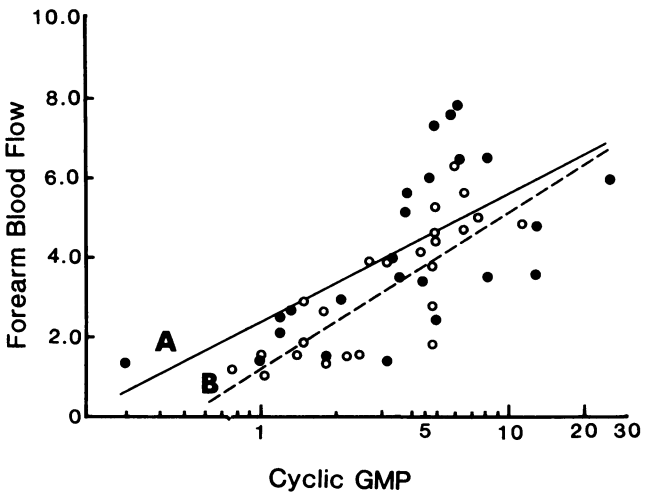

Figure 4. (*A*) Regression analysis of FBF (ml/100 ml per min) to ipsilateral venous plasma cyclic GMP concentrations (pmol/ml) during the infusions of αhANP at stepwise increasing doses of 20, 100, and 500 ng/min without $CaCl_2$ (*closed circles*) ($r = 0.637$, $P < 0.01$), and with the addition of isoosmolar $CaCl_2$ at a rate of 0.09 meq/min (*open circles*) ($r = 0.807$, $P < 0.01$). A straight line (*A*) denotes the regression line of the FBF-plasma cyclic GMP relationship during infusions of αhANP without $CaCl_2$; a dotted line (*B*), the regression line during the simultaneous infusions of αhANP and $CaCl_2$. Note that there was no significant difference in the slope between these two regression lines of the FBF-plasma cyclic GMP relationship.

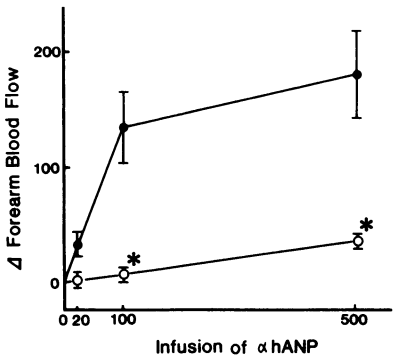

Figure 5. Changes (mean±SEM) in FBF (%) during the infusions of the stepwise increasing doses of αhANP with (*open circles*) and without (*closed circles*) the simultaneous infusion of $CaCl_2$. *$P < 0.05$ vs. the respective value without $CaCl_2$.

Throughout the study, systemic blood pressure and heart rate did not change. Contralateral venous plasma cyclic GMP did not change during infusions of αhANP (Table III).

Effects of local calcium infusion on FBF and plasma cyclic GMP concentration. During the infusion of $CaCl_2$, FBF was significantly decreased from 3.11±0.59 to 2.27±0.52 ml/100 ml per min (NS) at 5 min of $CaCl_2$ infusion, to 2.10±0.36 ml/100 ml/min ($P < 0.05$) at 10 min of the infusion, and to 2.09±0.46 ml/100 ml/min ($P < 0.05$) at 20 min of $CaCl_2$

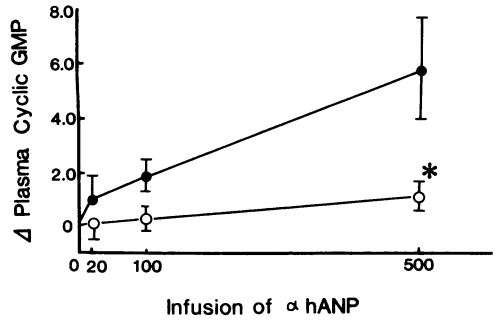

Figure 6. Changes (mean±SEM) in ipsilateral venous plasma cyclic GMP (pmol/ml) during the infusions of αhANP at the stepwise increasing doses of 20, 100, and 500 ng/min with (*open circles*) and without (*closed circles*) the simultaneous infusion of $CaCl_2$. *$P < 0.05$ vs. the value during the infusion of 500 ng/min αhANP without $CaCl_2$.

Table III. Systemic and Forearm Hemodynamics, and Biochemical Parameters during αhANP Infusion at a Rate of 50 ng/min before, during, and after the Simultaneous Infusion of $CaCl_2$ at a Rate of 0.09 meq/min in Five Normal Subjects (Mean±SEM)

	Control-1*	ANP 50	Control-2	Ca	Ca + ANP 50	Control-3	ANP 50
Blood pressure, *mmHg*							
Systolic	124±5	122±5	122±4	122±3	122±4	122±4	121±4
Diastolic	70±3	69±3	70±3	70±3	69±3	69±3	69±3
Heart rate, *bpm*	66±2	66±2	65±3	64±2	64±2	65±2	64±2
FBF, *ml/100 ml/min*	2.89±0.75	4.21±0.80‡	2.60±0.53	1.62±0.39	1.78±0.28	2.33±0.50	3.53±0.71§
FVR, *U*	42.2±8.1	26.3±7.4‡	42.0±10.1	66.4±13.0	57.2±12.2	50.3±14.3	34.5±11.8§
Ipsilateral cGMP	3.20±0.44	5.10±0.82‡	4.31±0.84	3.90±0.94	4.70±0.85	4.20±0.78	5.84±0.67§
Contralateral cGMP	3.10±0.32	3.23±0.54	3.21±0.64	3.10±0.61	3.23±0.43	3.21±0.41	3.35±0.46
Ipsilateral Ca, *mg/dl*	9.4±0.2	9.3±0.2	9.1±0.2	11.3±0.3‖	11.9±0.3‖	9.4±0.2	9.4±0.1
Contralateral Ca, *mg/dl*	9.3±0.1	9.2±0.3	9.2±0.2	9.2±0.1	9.4±0.1	9.4±0.1	9.3±0.1

* Control-1 and control-2, during the infusions of 5% dextrose; ANP 50, during the infusion of isosmolar $CaCl_2$ (0.09 meq/min); Ca + ANP 50, during the infusion of 0.09 meq/min $CaCl_2$ and 20 ng/min αhANP. ‡ $P < 0.05$ vs. the value of control-1. § $P < 0.05$ vs. the value of control-3. ‖ $P < 0.01$ vs. the value of control-1.

infusion (Table IV). At 10 min after switching the $CaCl_2$ infusion to 5% dextrose infusion, FBF restored toward normal: 2.86±0.61 ml/100 ml per min. In contrast to the $CaCl_2$-induced decrease in FBF, plasma cyclic GMP concentrations did not change throughout the infusion of $CaCl_2$.

Discussion

The results of the present studies indicate that in man intraarterial infusions of synthetic αhANP decrease FVR and increase FBF in a dose-related fashion. Thus, αhANP elicits a concentration-dependent vasodilation of forearm vascular beds. Previous studies showed that pronounced natriuresis and diuresis and a mild hypotension occurred when αhANP was intravenously injected in animals (1–6) and man (12–20). The depressor actions of synthetic αhANP were thought to be mediated through vasodilation, since several investigators have demonstrated that ANP are potent relaxants of norepinephrine-constricted aortic-strips or are dilators of renal blood vessels in isolated perfused rat kidneys that are constricted by norepinephrine (6, 44). However, the continuous intravenous infusion of synthetic ANP in conscious rats failed to decrease systemic and regional vascular resistances, rather accompanied by the increase in vascular resistances and the decrease in cardiac output (23, 24), although in anesthetized rats the administration of ANP decreased systemic and regional vascular resistances (21, 22). Thus, in conscious rats these vascular resistances were not decreased, possibly through reflex compensatory mechanisms, in order to maintain arterial pressure in the face of decreased cardiac output (23). Similar hemodynamic changes with infusions of αhANP have been recently demonstrated in man; the intravenous infusion of αhANP decreased blood pressure, associated with a moderate decrease in renal blood flow (19, 20). Because it is devoid of circulatory reflexes, in the present study αhANP was infused into the brachial artery at doses so low that they did not alter heart rate or systemic arterial pressure, and so we could evaluate direct pharmacological actions of αhANP on the forearm vascular beds in man. Thus, it suggests that αhANP elicits a pronounced vascular relaxant effect in man, which is consistent with the result of the recent study using a similar intraarterial model (45).

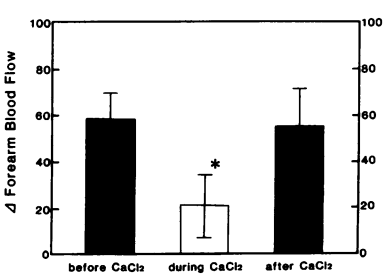

Figure 7. Changes in FBF (%) with infusions of 50 ng/min αhANP, before, during, and after the simultaneous infusion of $CaCl_2$ (mean±SEM). *$P < 0.05$ vs. both before $CaCl_2$ infusion and after the discontinuation of $CaCl_2$ infusion.

A significant positive linear correlation between level of initial limb vascular resistance and magnitude of limb vascular response to vasodilator agents has been reported (25). In the present study there was a similar significant correlation between initial resistance and magnitude of dilator response to αhANP. The fact that the assumption of linearity was correct for the case of αhANP is consistent with the hypothesis that αhANP is a true vasodilator agent.

The second important observation is that intrabrachial infusion of αhANP at the stepwise increasing rates induced the dose-dependent increase in ipsilateral venous plasma cyclic GMP, indicating αhANP-dependent cellular cyclic GMP formation. In the present study, moreover, there appeared to be an intimate relationship between the increase in cyclic GMP production and the vascular relaxation induced by αhANP, since there was a significant positive correlation between FBF and ipsilateral venous plasma levels of cyclic GMP during infusions of αhANP. It is consistent with the result of in vitro studies that αhANP caused a time-dependent and concentration-dependent increase in tissue levels of cyclic GMP that corresponded with vascular relaxation in the same vascular smooth muscle of the rabbit thoracic aorta (29). Thus, cyclic GMP is thought to mediate vascular relaxation induced by ANP (26–31). Furthermore, the increase in cellular cyclic GMP formation is apparently due to an ANP-induced activation of particulate guanylate cyclase rather than to an inhibition of phosphodiesterases, since ANP-induced vascular relaxation and elevation of cyclic GMP levels could be inhibited by

Table IV. Effects of $CaCl_2$ Infusions (0.09 meq/min) on FBF and Plasma Cyclic GMP (Mean±SEM)

	Control*	Ca-5 min	Ca-10 min	Ca-20 min	Recovery
FBF, *ml/100 ml/min*	3.11±0.59	2.27±0.52	2.10±0.36‡	2.09±0.46‡	2.86±0.61
Ipsilateral cGMP, *pmol/ml*	4.83±1.04	ND	4.26±0.68	4.59±0.92	4.80±0.96
Contralateral cGMP	4.94±1.27	ND	4.56±0.88	4.63±0.99	4.62±0.99

* Control, during the infusion of 5% dextrose; Ca-5 min, at 5 min of $CaCl_2$ infusion; Ca-10 min, at 10 min of $CaCl_2$ infusion; Ca-20 min, at 20 min of $CaCl_2$ infusion; recovery, 10 min after the discontinuation of $CaCl_2$ infusion. ‡ $P < 0.05$ vs. control.

the guanylate cyclase inhibitor methylene blue, but not inhibited by the phosphodiesterase inhibitor M&B 22,948 (27, 28, 30). Supporting this hypothesis, Garcia et al. have demonstrated that lack of Ca^{2+} in the extracellular medium did not prevent the relaxing effect of ANP on rat renal arterial strips previously made to contract by application of norepinephrine or angiotensin II (46). Moreover, the vasorelaxant effect of ANP in isolated kidneys perfused in the presence of vasoconstrictors is at least partially independent of extracellular Ca^{2+} (47). These findings suggest that the effect of ANP is not mediated by an impairment of Ca^{2+} influx from the extracellular medium. In addition, Winquist et al. have suggested that ANP increase cyclic GMP independently of extracellular Ca^{2+}, since the ANP-induced increases in cyclic GMP were observed in isolated rabbit aortic segments pretreated with calcium-free buffer (28). Thus, similarly to other vasorelaxants such as nitroprusside, it is possible that cyclic GMP is the second messenger of the ANP-induced vasorelaxant effect (48). This hypothesis is supported by the results of the present study that the $CaCl_2$-induced inhibition of the ANP-dependent vascular relaxation was accompanied by the attenuation of increases in plasma cyclic GMP, which might reflect cellular cyclic GMP formation in vascular smooth muscle.

Regarding high extracellular Ca^{2+} effects on the vascular actions of αhANP, in the present study one should consider the down regulatory mechanisms (49); the blunted vascular response to the infusion of αhANP with $CaCl_2$ might be influenced by the preceding infusion of αhANP. However, ipsilateral venous plasma αhANP concentrations immediately before the second infusion of αhANP with $CaCl_2$ were almost same as those before the first infusion of αhANP without $CaCl_2$. Finally, after the discontinuation of the αhANP and $CaCl_2$ infusion these blunted responses of vasodilation and cyclic GMP formation to αhANP infusion were restored to normal. Therefore, high Ca^{2+} effects on the vascular actions of αhANP should not be explained by "down regulation" of vascular αhANP receptors but be attributed to the action of Ca^{2+} per se. Since Ca^{2+} has a direct effect upon insulin receptor binding and affinity (50), there are some possibilities that high Ca^{2+} may change the affinity of αhANP receptors, which have a dissociation constant consistent with the concentration of αhANP that causes cyclic GMP formation and vasorelaxation (51), and thus attenuate the αhANP-induced increase in cellular cyclic GMP production and the vascular relaxation. However, there is no evidence about the modulating effect of extracellular Ca^{2+} on vascular ANP-receptor.

It is established that Ca^{2+} is an important regulator of hormone-sensitive adenylate cyclase and guanylate cyclase (32). Several investigators have described that high concentration of extracellular Ca^{2+} sharply inhibited particulate guanylate cyclase activity in the heart and lung (35, 36). Moreover, high Ca^{2+} blunted the action of antidiuretic hormone, by inhibition of adenylate cyclase activation in the renal tubules (33, 34). Therefore, it is suggested that high Ca^{2+} might suppress αhANP-dependent cyclic GMP production in the smooth muscle cell, through the inhibition of particulate guanylate cyclase activation, although high Ca^{2+} did not change basal plasma cyclic GMP levels. Other possibilities may include the stimulation of the cyclic GMP hydrolysis, through the Ca^{2+}-activated cyclic nucleotide phosphodiesterase (52).

Regarding the αhANP-induced vasorelaxation, alternative explanations are not ruled out, including the possibility that the apparently close association between αhANP-elicited cyclic GMP production and relaxation is merely fortuitous. Haass et al. have recently suggested that in the pithed, vagotomized rat ANP resemble the calcium channel blocker nifedipine rather than the general vasodilator sodium nitroprusside, and attenuate the slow, calcium flux dependent component of alpha adrenoreceptor-mediated pressor responses (53). Thus, it is possible that αhANP relax vascular smooth muscle, by inhibiting an influx of extracellular calcium rather than by the increased cyclic GMP production.

In the present study, infusion of $CaCl_2$ alone caused vasoconstriction without changes in plasma cyclic GMP, suggesting that the mechanism for Ca^{2+}-induced vasconstriction might not involve the vascular cyclic GMP systems. Although increased Ca^{2+} in medium could relax arterial strips (54), intraarterial infusion of calcium produced vasoconstriction in man (55). Regarding Ca^{2+}-induced vasoconstriction, in the presence of circulating vasoconstrictors such as norepinephrine and angiotensin the increase in extracellular calcium concentration may augment calcium entry into cell through receptor-operated calcium channels, and thus, constricting the vascular beds by the increased intracellular calcium concentrations. Moreover, it has been demonstrated that a relatively high extracellular Ca^{2+} inhibits specific calcium channels in vascular smooth muscle cells, possibly by the augmented inactivation of calcium channels, and then antagonizes the inhibitory effect of the calcium antagonists, cobalt (56) and magnesium ions (57). In this study, therefore, it is suggested that the increased Ca^{2+} might inhibit the ANP-dependent calcium channels, and thus attenuate the decrease in intracellular Ca^{2+}, leading to the blunted ANP-dependent vasodilation. However, there is no evidence about Ca^{2+} effects on the inactivation of ANP-dependent calcium channels.

An intricate relationship between cyclic nucleotide-mediated events and changes in the activity of different Ca^{2+} translocating mechanisms is likely to be important in most excitatory tissues. Ca^{2+} regulates cyclic nucleotides metabolism in several ways, conversely cyclic nucleotides can regulate

calcium metabolism in either a positive or negative way (32). Therefore, there are some possibilities that pretreatment with calcium blunted the vascular relaxant effect of αhANP, through the interaction of cyclic nucleotides and intracellular Ca^{2+}. It should be noted, however, that in the present study modest elevations of serum calcium could attenuate the vascular responses to αhANP, whereas most of the above-mentioned in vitro studies concerned the effects of calcium at clearly superphysiological level. Further studies are needed to clarify these issues.

In summary, synthetic αhANP was infused into the brachial arterial blood in healthy volunteers, because it was devoid of circulatory reflexes. The intrabrachial infusion of αhANP caused a dose-dependent vasodilation of forearm vascular beds, with the concomitant increase in ipsilateral venous plasma cyclic GMP concentrations. There appeared to be a significant positive linear relationship between the cyclic GMP increase and the vascular relaxation induced by αhANP. Moreover, the simultaneous infusion of $CaCl_2$ not only inhibited the αhANP-induced vascular relaxation but also blunted the accompaning increase in cyclic GMP levels. Therefore, it is suggested that αhANP acts directly on the forearm vascular beds in man, eliciting its vascular relaxant effect, possibly by increasing cellular cyclic GMP formation. Moreover, high extracellular calcium inhibits the αhANP-induced vasodilation, possibly through the suppression of cyclic GMP increase.

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